APPENDIX A

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Review

Thymulin and the neuroendocrine system

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Abstract

Thymulin is a thymic hormone exclusively produced by the thymic epithelial cells. It consists of a nonapeptide component coupled to the ion zinc, which confers biological activity to this molecule. After its discovery in the early 1970, thymulin was characterized as a thymic hormone involved in several aspects of intra- and extrathymic T-cell differentiation. Subsequently, it was demonstrated that thymulin production and secretion is strongly influenced by the neuroendocrine system. Conversely, an emerging core of information points to thymulin as a hypophysotropic peptide. Here we review the evidence supporting the hypothesis that thymulin is an important player in the hypophyso-thymic axis.

Keywords: Thymulin; Hypophysiotropic activities; Nude mouse; Aging; Dyshomeostasis; Neuroendocrine imbalances; Gene therapy

1. Introduction

It is now well-established that the immune system is functionally linked to the nervous and endocrine systems thus constituting an integrated homeostatic network [23]. Within this network, the neuroendocrine system monitors and controls the physical and chemical variables of the internal milieu. On its part, the immune system perceives, through antigenic recognition, an internal image of the macromolecular and cellular components of the body and reacts to alterations of this image, effectively participating of the "biological" homeostasis of the organism. The relevance of the thymus in this network becomes evident when one observes the immune and neuroendocrine consequences of neonatal thymectomy or congenital absence of the thymus in certain animal species (see below). In this context, the existence of a neuroendocrine-thymic axis is well-documented (for a review, see [47]).

Thymulin is a thymic hormone involved in several aspects of intra- and extrathymic T-cell differentiation [1]. Thymulin, which is exclusively produced by the thymic epithelial cells (TEC), consists of a biologically inactive nonapeptide component (facteur thymique sérique or FTS) coupled in an equimolecular ratio to the ion zinc [20], which confers biological activity to this molecule [13]. In the present paper, we will review the evidence indicating that thymulin is an important player in the hypophyso-thymic axis.

2. Evidence for a pituitary-thymulin axis

The control of thymulin secretion seems to be dependent on a complex network of events. Initial studies showed that the hormone itself exerts a controlling feedback effect on its own secretion both in vivo and in vitro [9,48].

Additionally, thymulin production and secretion is influenced directly or indirectly by the neuroendocrine system. For instance, growth hormone (GH) can influence thymulin synthesis and secretion. In vitro, hGH can stimulate thymulin release from TEC lines [50] which are known to possess specific receptors for GH [2]. Animal studies have shown that treatment of aged dogs with bovine GH partially restored their low thymulin serum levels [21]. In old mice, treatment with ovine GH increased their low circulating thymulin and enhanced the concanavalin A (Con A)-dependent proliferative response of thymocytes, as well as interleukin-6 production [26]. In old rats, combined treatment with GH and thyroxine (T_4) was also able to restore partially their reduced thymulin levels [27]. In clinical studies, it was reported that in congenitally GH-deficient children, who consistently exhibited low plasma thymulin levels, GH therapy succeeded in increasing thymic hormone levels to near normal values [41]. Acromegalic middle aged patients have elevated thymulin serum levels compared to age-matched normal sub-

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jects [41,50]. It is likely that these effects of GH are mediated, at least in part, by insulin-like growth factor 1 (IGF-1) as suggested by the fact that the GH-induced enhancement of thymulin production in vitro could be prevented by previous treatment with antibodies against IGF-1 or IGF-1 receptor [50].

There is also evidence for a prolactin (PRL)-thymulin axis. Thus, it is known that TEC possess PRL receptors [12] and that PRL can stimulate thymulin synthesis and secretion both in vitro and in vivo [15]. Furthermore, administration of PRL to old mice elevated their reduced circulating levels of thymulin [15].

The thyroid axis also influences thymulin secretion. Thus, T_4 has been shown to stimulate thymulin synthesis and secretion in mice [17]. In vivo treatment of mice with triiodothyronine (T_3) enhanced thymulin secretion whereas treatment of the animals with propylthiouracil, an inhibitor of thyroid hormone synthesis, decreased their circulating thymulin levels [49]. In humans, hyperthyroidism brings about an increase in circulating thymulin levels whereas hypothyroid patients show depressed levels of this thymic hormone [18]. In in vitro studies, it was shown that thyroid hormones stimulate thymulin secretion by a direct action on TEC [40,51]. Interestingly, it has been shown that treatment of aged animals with T_4 can reverse their decreased thymulin levels [17,40].

Although there are no studies documenting a direct effect of gonadotropins or adrenocorticotropic hormone (ACTH) on thymulin secretion, gonadectomy or adrenalectomy in mice are known to induce a transient decrease in serum thymulin levels. This effect is potentiated by the simultaneous removal of the adrenals and gonads [14]. In TEC cultures, it was shown that exposure to physiological levels of glucocorticoids or gonadal steroids enhanced thymulin concentration in the cell supernatants [46].

Although there is no rigorous evidence proving the existence of hypothalamic factors able to influence thymulin production by a direct action on TEC, there are two studies which suggest that this may be the case. Treatment of old mice with hypothalamic extracts from young mice resulted in reappearance of detectable levels of circulating thymulin [19]. Hypothalamic and pituitary extracts from young mice stimulated thymulin release from TEC cultures but this stimulation declined when the pituitary and hypothalamic extracts were obtained from old mice [28].

3. Hypophysiotropic activity of thymulin

The multilateral influence that the neuroendocrine system exerts on thymulin secretion suggests that this metallopeptide could in turn be part of a feedback loop acting on neuroendocrine structures. This possibility is now supported by a significant body of evidence indicating that thymulin possesses hypophysiotropic activity. Thus, thymulin has been shown to stimulate luteinizing hormone (LH) release from perifused rat pituitaries [53] and ACTH from incubated rat pituitary fragments, the latter being an effect mediated by intracellular cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) accumulation [31]. Thymulin has been found to stimulate GH, PRL, thyrotropin (TSH) and gonadotropin release in dispersed rat pituitary cells at doses from 10^{-8} to 10^{-3} M [4–6] whereas others have reported that thymulin doses of 10^{-11} M stimulate LH, inhibit PRL release and have no effect on GH secretion in incubated rat pituitary fragments [31]. The stimulatory effect of thymulin on hormone release in rat pituitary cells declines with the age of the cell donor which suggests that aging brings about a desensitization of the pituitary gland to thymic signals [4–6].

Interestingly, congenitally athymic (nude) mice display a number of neuroendocrine deficiencies consistent with their lack of circulating thymulin (i.e., while thymulin generally stimulates pituitary hormone secretion, athymia seems to be associated with a functional panhypopituitarism). Thus, in a series of studies it was demonstrated that in nude CD-1 male mice, TSH, PRL, GH and gonadotropin responses to immobilization and cold stress are reduced as also are serum basal levels of the same hormones [25,29,30]. Nude female mice show significantly reduced levels of circulating and pituitary gonadotropins, a fact that seems to be causally related to a number of reproductive derangements described in these mutants [43]. Thus, in homozygous (nu/nu) females the times of vaginal opening and first ovulation are delayed [3], fertility is reduced [43], and follicular atresia is increased such that premature ovarian failure results [37]. Similar abnormalities result from neonatal thymectomy of normal female mice [39,42]. There is in vitro and in vivo evidence suggesting that thymulin plays a role in the regulation of female spontaneous puberty, possibly through its effects on ovarian steroidogenesis [32]. Thymulin also modulates gonadotropin-induced testicular steroidogenesis [52].

A functional impairment of the hypothalamo-adrenal axis has been reported in nude mice suggesting that humoral thymic factors may play a role in the physiology of this axis [11].

Since thymulin has been shown to be active on the nervous system (for a review, see [45]) and hypothalamic factors appear to influence thymulin secretion (see above), the peptide might exert a direct or indirect feedback effect at hypothalamic level.

4. The prospect of gene therapy for thymulin in thymus-depressing pathologies

Studies in animals have demonstrated that aging brings about a severe involution of the thymus and that circulating levels of thymulin are very low in old animals [21,26,27]. In healthy humans, serum thymulin levels remain high until 10–15 years of age, then fall progressively until 35–40 years of age, remaining at very low plateau levels afterwards

[10,16,35]. There is also a number of clinical situations associated with markedly low levels of circulating thymulin. These situations include, but are not limited to, AIDS [34], Di George syndrome [35] and other immunodeficiencies [33,35] as well as Down's syndrome [16].

As thymus involution is very difficult to reverse by pharmacological means, the prospect of implementing thymic hormone gene therapy appears as an interesting avenue of research aimed at restoring circulating levels of thymic hormones when thymus function is compromised [24]. Unfortunately, up to date the gene coding for thymulin has not been cloned, a situation that hinders the application of gene therapy for this thymic hormone. A possible way to overcome this problem is to construct an "artificial gene" coding for thymulin. Previous studies demonstrated that this can be done in bacteria. Thus, a synthetic DNA sequence coding for thymulin was inserted into a bacterial expression vector and successfully used to obtain large quantities of purified thymulin retaining full biological activity [8]. Consequently, an adenoviral vector (adenoviral vectors are highly efficient gene delivery systems) harboring this synthetic gene for thymulin could be constructed. As the synthetic gene product must be targeted to the secretory pathway, so that thymulin may be secreted by the transduced cells, a secretory signal DNA sequence would have to be attached upstream the synthetic gene. This type of strategy has been successfully implemented for other proteins [36].

Skeletal muscle has been shown to be a well-suited tissue for efficient viral-vector-mediated peptide hormone gene transfer as well as for long-term regulated expression and secretion of the transgenic peptide [38,44]. Therefore, the adenoviral vector carrying the thymulin-encoding DNA sequence could be injected intramuscularly in an appropriate animal model as the nude mouse. Transduced myocytes should then begin to act as an ectopic source of thymulin thus restoring circulating thymulin levels. Additionally, the thymulin synthetic gene carried by the adenoviral vector could be placed under the control of a regulable promoter. That is, a promoter that can be turned on or off by the administration of small molecules like the antibiotic tetracycline or the steroid mifepristone, both of which have been successfully used for this purpose [7,22]. The use of a regulable vector system for the thymulin synthetic gene would allow to control the circulating levels of the hormone, lowering or increasing them as physiological or pathological circumstances demand.

5. Concluding remarks

While the existence of a neuroendocrine-thymic axis is well-established, the evidence reviewed here strongly suggests that thymulin plays a physiological role as part of an ascending feedback loop in this axis. Consequently, it would be reasonable to expect that pathological situations associated with thymulin deficiency (or overproduction) may alter the neuroendocrine balance. Within this context, gene therapy for thymulin may succeed in restoring some of the immune, endocrine, and reproductive abnormalities associated with thymus deficiency.

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